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Multimorbidity in vitiligo patients-emphasis on autoimmune diseases and involvement of immune response regulatory genes

U. Yu Sabirov[1,*], A. Sh Inoyatov[1], D. R. Xakimov[1] and J. Sh Narziyev[1]

[1] Republican Specialized Scientific and Practical Medical Center of Dermatovenerology and Cosmetology of the Ministry of Health of the Republic of Uzbekistan, 100109, Tashkent, Uzbekistan.

* Correspondence: sabirovu@gmail.com

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Abstract: The pathogenesis of autoimmune diseases (AD) is based on the "recognition of the body's own structures by immunocompetent cells and subsequent activation, proliferation, and induction of inflammation. Most lymphocytes directed towards their own antigens are removed in the thymus through apoptosis." Currently, research is being conducted to investigate the genes involved in the development of autoimmune diseases, specifically in the context of vitiligo, such as HLA genes (HLADR3 associated with a common process, HLADR4 with limited), TNFAIP3 genes, and A20 deficiency haplotype (a product of this gene). The importance of immune inflammation, mononuclear infiltration of marginal zones, and the cytotoxic effect of CD8+ on melanocyte changes in CD4, CD8, and their ratios have been identified as early indicators of vitiligo progression. A local deficiency of SOD, glutathione, and scavengers from ROS regulated through Nrf2 and ARES in the skin and blood of vitiligo patients has also been revealed. This study included 287 patients with non-segmental vitiligo (148 men and 139 women) aged 19-68 years who sought treatment at the Republican Specialized Scientific and Practical Medical Center of Dermatovenerology and Cosmetology between 2018 and 2022. The average age was 28.1±1.3 years, and the duration of the disease was 42.6±3.6 months. Of these patients, 114 (40%) had the debut of vitiligo less than a year before treatment. The study found that the average level of TNF-alpha was higher than the reference interval and the data of the control group, supporting the contribution of inflammation and apoptosis to depigmentation in vitiligo. The study also highlighted the levels of TNF-alpha and IL-21 in patients who had an unfavorable allele for the SNPs among the other patients in the main group.

Keywords: Vitiligo; Autoimmune process; Gene; Mutation.

1. Introduction

Epidemiological analysis conducted in the European population in Europe and America for 62 years (1951-2013) indicates a "bimodal distribution of the onset of vitiligo, which is characteristic of autoimmune diseases (type 1 diabetes, juvenile rheumatoid arthritis and rheumatoid arthritis, SLE)" [25]. Based on a retrospective analysis of 4406 patients over the specified time period, it was found that "the average age of debut for the specified period doubled, and the change was most pronounced from 1973 and 2000, so, until 1970. the onset of the disease occurred at the age of 14.6 ± 9.4 years, the age of vitiligo onset increased by about four months per year and stabilized only in 2004, amounting to 30.2 ± 17.3 years" [1]. Since genetics has not changed, the age shift of the onset of the disease indicated that since 1973 and 2000, either the impact of trigger factors has decreased, or the biological response to them has changed [2,3].

Vitiligo is significantly more often observed in people with concomitant autoimmune diseases - nest allopecia, DM-1, Adisson's disease, thyroiditis, and the pathogenesis of autoimmune diseases (AD) is based on "recognition of the body's own structures by immunocompetent cells and their subsequent activation, proliferation and induction of inflammation; most of the lymphocytes directed to their own antigens are removed in the thymus as a result of apoptosis, but some avoid this process and are in the peripheral

lymphatic tissue, remaining intact naive, inactive" [4,5]. Currently, work is underway to study the genes involved in the implementation of autoimmune diseases in the aspect of the relationship with vitiligo, in particular HLA genes (HLADR3 is associated with a common process, HLADR4 - c limited), TNFAIP3 genes and A20 deficiency haplotype (a product of this gene) (Clinical Genetics Centre, Japan), the importance of immune inflammation is shown, mononuclear infiltration of marginal zones and cytotoxic effect of CD8+ on melanocytes in the central depigmentation zone (Hospital Saint Andre in Bordeaux, France); the role of cytokines - IL2,IL-3, IL-4, IFN-gamma, TNF-alpha, granulocyte- and-macrophage colony-stimulating factor in the development of depigmentation (Cutis Academy of Cutaneous Scientist in Bangalore, India) was proved; changes in CD4, CD8, and their ratios as early markers of progressive course were established vitiligo (Medical College in Raipur, India); revealed a local deficiency of SOD, glutathione and scavengers from ROS regulated through Nrf2 and ARES in the skin and blood of vitiligo patients (University of Turin, Italy) [2,5–7]. The autoimmune theory of vitiligo is dominant, when the death of melanocytes is carried out due to their attack by autoantibodies, as well as melanocyte-specific cytotoxic CD8+. Morphologically, the deposition of the C3 component of the complement and the accumulation of Langerhans cells at the level of the basal layer of the epidermis is noted [8]. It should be emphasized that Langerhans cells, having surface receptors for the Fc fragment of the IgG3b complement, contain antigens encoded by immune response genes and, therefore, carry out immunological "supervision". Autoaggression to melanocytes can cause their death with the development of depigmentation. The cytokines associated with the development of AIZ are TNF-alpha, interferons alpha and beta [6,7]. The realization of the effects of TNF depends on the state and expression of its receptors, as well as on its negative regulator - A20, the product of the TNFAIP3 gene [9]. A20 is also a negative regulator of the NFkB signaling pathway [10]. Heterozygous mutation in this gene is observed only in autoimmune diseases - Behcet's disease [11]. The most common clinical symptoms in the form of fever in persons with such a mutation (haploinsufficiency A20) develop in Asians, and their clinical manifestations from the skin are erased, whereas in Europeans and residents of the USA they are more vivid; at the same time, in Asians, the use of TNF-alpha, IL-1 and Janus kinase inhibitors is more effective [11]. Recently, in the light of the autoimmune theory of the development of vitiligo, the appointment is recommended and the effect of local and systemic use of immunosuppressants - tacrolimus, mycophenolates in vitiligo has been obtained [12–14]. Based on the autoimmune theory of vitiligo development, which has been preferred recently, it is of interest to study cytokines associated with AIZ, as well as their genes and genes involved in the realization of the effect of these cytokines. From these positions, it is of interest to study the genes REL, IL-21, as well as the genes of anti-TNFa - TNFAIP3.

The purpose of this research is to look at the structure of concurrent somatic illnesses in people with vitiligo, as well as single-nucleotide polymorphisms in the TNFAIP3 and REL genes, which are implicated in the development of autoimmune disorders.

2. Materials and Methods

There were 287 patients with non-segmental vitiligo (148 men and 139 women) aged 19-68 years who applied to the Republican Specialized Scientific and Practical Medical Center of Dermatovenerology and Cosmetology in the period from 2018 to 2022, the average age was 28.1±1.3 years. All had non-segmental vitiligo, the duration of the disease was 42.6±3.6 months, while 114 patients (40%) had the debut of vitiligo for less than 1 year before treatment.

Molecular genetic studies were carried out on the basis of the laboratory "Kani-Med Healthcare" within the framework of the agreement on scientific cooperation. 20 patients with vitiligo of the main group and 40 patients of the population control group were examined, because they were of the same race, were born in Uzbekistan, lived in Tashkent. Single nucleotide polymorphism (SNP) of 2 candidate genes was studied: TNFAIP3 gene - rs2230926 (F127C), rs753955178) (P461P), rs146534657 (N102S); REL gene - rs142878172 (IVS10-7C>A), rs140572082 (T433A). The calculation of the adequacy of the sample and the distribution of allele variants of the trait was checked using the Hardy-Weinberg law according to the equation p2 +2pq+q2=1. Where p is the frequency of the dominant trait (wild type), q is the frequency of the recessive trait (mutant type), expressed in fractions of one. Databases were also used to establish the general population frequency.

3. Results and discussion

The study of anamnestic data showed that the onset of the disease in 100 (35%) patients was associated with psycho-emotional stress, in 43 (15%) cases - with somatic pathology, family cases of vitiligo amounted to 63 (21.9%), persons with autoimmune diseases had a history of 26 (9.1%). In other cases, patients could not indicate the cause of the onset of the disease. Attention is drawn to the fact that autoimmune diseases (AIZ) in the anamnesis were named only 26 out of 287 patients with vitiligo, as well as helminthic invasions, although an in-depth examination revealed AIZ in 61 (21.2%) patients, helminthic invasion - in 42 (14.6%), the incidence of overweight and obesity was the same, and liver and biliary tract diseases, anemia, thyroid diseases, worm infestations and autoimmune diseases were significantly more common in people with vitiligo ($p<0.05$). Diseases of the respiratory organs, ENT organs, and genitourinary system were found in isolated cases in both groups. Diseases of the cardiovascular system in persons with vitiligo were represented by arterial hypertension, coronary artery disease, cardiomyopathy, which were somewhat more common than in the control group, although the differences were not statistically significant ($p>0.05$).

In general, concomitant diseases were found in 186 (64.8%) patients. In the control group, concomitant pathology was significantly less common - 47.5% versus 64.8% ($p<0.05$) (Table 1).

Table 1. Somatic diseases in patients with vitiligo

Factor	Main group, n=287		Control group, n=40		p	RR
	n	%	n	%		
Stressful situations at work/in the family	43	15,0	6	15,0	P>0,05	1,00
Family history of vitiligo	63	22,0	0	0,0	P<0,05	-
Autoimmune diseases (thyroiditis, RA, alopecia, DM-1)	61	21,3	1	2,5	P<0,05	10,53
Thyroid dysfunction	137	47,7	2	5,0	P<0,05	17,35
Obesity	19	6,6	2	5,0	p>0,05	1,35
Overweight	136	47,4	16	40,0	p>0,05	1,35
Type 2 diabetes mellitus	18	6,3	0	0,0	P<0,05	-
Arterial hypertension	29	10,1	3	7,5	p>0,05	1,39
Ischemic heart disease	10	3,5	1	2,5	p>0,05	1,41
Cardiomyopathy	1	0,3	0	0,0	p>0,05	-
Liver and gallbladder diseases	47	16,4	3	7,5	P<0,05	2,42
Diseases of Otolaryngology-organs	8	2,8	1	2,5	p>0,05	1,12
Respiratory diseases	22	7,7	2	5,0	p>0,05	1,58
Diseases of the kidneys and Urinary tract	12	4,2	1	2,5	p>0,05	1,70
Diseases of the reproductive system	9	3,1	1	2,5	p>0,05	1,26
Allergy	18	6,3	1	2,5	P<0,05	2,61
Skin diseases	16	5,6	2	5,0	p>0,05	1,12
Anemia	138	48,1	5	12,5	P<0,05	6,48
H.pyllori	16	5,6	2	5,0	p>0,05	1,12
Helminthic invasion (IgG Lamblia intestinalis, IgG Ascaris lumbricoides)	42	14,6	1	2,5	P<0,05	6,69
Fungal infection (IgG Candida albicans, IgG Helicobacter pillory, IgG Aspergillus fumigatus)	11	3,8	0	0,0	P<0,05	-
TOTAL concomitant diseases	186	64,8	19	47,5	P<0,05	2,04

As our observations have shown, in the structure of autoimmune diseases in persons with vitiligo, autoimmune thyroid damage with a high level of antithyroidism, manifestations of hypo- and hyperthyroidism prevailed, which was regarded as autoimmune thyroiditis in 56 (19.5%) patients, type 1 diabetes was in 2 (0.7%), RA was in 1 patient (0.3%), alopecia - in 2 (0.7%). At the same time, an increase in anti-TPO was in 69 (24.8%) patients, hypothyroidism was detected in 67 (23.3%), hyperthyroidism with an increase in T4 and T3 - in 137 (47.7%), a decrease in TSH - in 69 (24%). In 15 patients, the increase in anti-TPO was more than 10 times from the upper limit of the reference interval, reaching 385.5 IU/ml.

Thyroid dysfunction occurred in almost half of the patients with vitiligo, whereas in the control group only in 2 patients (5%), which is statistically significantly less frequent. The presence of helminthic invasion according to the results of ELISA analysis (parasitic complex IgG Lamblia intestinalis, IgG Ascaris lumbricoides) was detected in 42 (14.6%), antibodies to H.pillory - in 16 (5.6%), the presence of fungal infection (IgG Candida albicans, IgG Helicobacter pillory, IgG Aspergillus fumigatus) - in 11 (3.8%) patients of the main group.

The average values of thyroid status indicators in patients with vitiligo had a wide range of fluctuations, however, for the parameters of TSH, T4, anti-TPO, the differences were statistically significant at p<0.05 (Table 2).

Table 2. The level of T3, T4, anti-TPO, TSH in patients with vitiligo

Indicator	Reference interval	Control group	Main group	Reliability of differences from the control group
TSH, mcIU/ml	0,3-4,5	0,84±0,2	3,83± 1,24	P<0,05
free T3, pg/ml	2,0-4,2	2,84± 0,12	3,07± 0,99	P>0,05
free T4, pg/ml	8,9-17,2	15,1± 0,4	35,1± 2,39	P<0,05
Anti-TPO, IU/ml	Less than 30	5,7± 1,0	54,4± 9,87	P<0,05

The study of vitamin D status in patients with vitiligo showed its normal level only in 55 (19.2%) patients, vitamin D deficiency was in 103 (35.9%), deficiency of less than 20 ng/ml - in 129 (44.9%) patients. At the same time, in the control group, vitamin D deficiency was in 3 (7.5%), deficiency - in 4 (10%) individuals, which is significantly less frequent than in patients with vitiligo (p<0.05). The average level of 25-OND3 was 24.1±1.5, whereas in the control group it was 32.0±0.3 ng/ml.

Anemia in vitiligo patients was significantly more common than in the control group (48.1% vs. 12.5%, p<0.05), although the average values of hemoglobin and erythrocytes in the group were 131.3±1.02 g/l and 5.47±0.98, respectively.

Also, 97 (33.8%) patients of the main group had dyslipidemia in the form of hypertriglyceridemia, hypercholesterolemia, although the average values of total cholesterol and TG did not significantly differ between the groups. Hyperglycemia in combination with an increase in glycated hemoglobin occurred in 20 (6.9%) patients of the main group who had DM-2 (n=18) and DM-1 type (n=2), 47 (16.4%) had hyperglycemia in the range of up to 7 mmol/l, indicating glucose tolerance disorders, as well as stress hyperglycemia.

The study of single-nucleotide polymorphisms of candidate genes involved in autoimmune diseases in patients with vitiligo showed that there were no deviations in the expected and observed frequencies of the distribution of alleles of genotypes of polymorphism of candidate genes according to the distribution of the Hardy-Weinberg law, with the exception of (rs140572082) T433A of the REL gene, for which there was no mutant in any case in the control group. variants of the allele. This fact requires further study, taking into account the fact that the population frequency of this SNP is less than 0.0005.

We should note that if an allele occurs in a healthy population with a high frequency, then it can be considered benign or probably benign (frequency = 0.1-0.01), if it occurs less frequently - with a frequency of 0.001, then its significance for pathology is not specified, if with a frequency of 0.0001, then it is likely pathogenic, and if with a frequency of 0.00001 - clearly pathogenic, carrying it leads to the death of the organism.

Of the selected genes and their polymorphisms, according to the TOPMED, GnomAD, dbSNP databases, the mutant G allele for rs2230926 (F127C) TNFAIP3 gene was probably benign, the mutant G allele for rs753955178 (P461P) TNFAIP3 gene was with unknown significance, the mutant G allele rs146534657 (N102S) TNFAIP3 gene was also with unspecified significance, the mutant allele A rs142878172 (IVS10-7C>A) of the REL gene - with unspecified significance, and the mutant allele G rs140572082 (T433A) of the REL gene - was likely pathogenic, because it is not characteristic of the population [15]. Perhaps this is the reason for his absence from the control group. At the same time, the presence of this allele in patients with vitiligo and the significance of the allele in the development of this disease requires clarification.

In general, as our studies have shown, G alleles for rs2230926 (F127C) TNFAIP3 gene with RR = 6.4 were at risk for vitiligo; mutant allele G rs753955178 (P461P) TNFAIP3 gene at RR =4.15; mutant allele G for rs146534657 (N102S) TNFAIP3 gene at RR =4.15; mutant allele A for rs142878172 (IVS10-7C>A) of the REL gene at RR =4.15. That is, the study of single-nucleotide polymorphisms of TNF-alpha TNFAIP3 reverse regulator genes, the product of which has a protective effect, revealed that vitiligo patients had mutant alleles for all 3 studied polymorphisms for the development of this pathology (Table 3).

Discussing the data obtained, we note that the TNFAIP3 rs2230926 gene is located on chromosome 6 (chr6:137874929 (GRCh38.p13)), this polymorphism is a missense variant of the TNFAIP3 gene and has allelic variants T>C/T>G. T is a wild allele, the mutant variant is G or C. The population frequency of the unfavorable

G allele according to various information sources varies from 0.04 to 0.14: G=0.046627 (16595/355906, ALFA), G=0.140202 (37110/264690, TOPMED), G=0.134318 (18826/140160, GnomAD). The distribution of this allele in different ethnic groups also varies, occurring more often in Africans, amounting to almost 45% (G=0.451), less often in Asians, amounting to about 4.4% (G=0.0437) [15].

In our observations, the frequency of the mutant allele G of the TNFAIP3 rs2230926 gene was 7.5% in the main group and 1.25% in the control group (χ2= 3.86; p<0.05), which indicates its presence in the studied healthy Uzbek population and a significant increase in vitiligo at RR=6.4.

Various polymorphisms of this gene, including SNP TNFAIP3 Cys243Tyr and Glu192Lys were detected in the Japanese population, and SNP Met476Ile was detected in the Chinese, Arg183X - in East Asia and other regions [11]. Currently, it has been convincingly shown that SNP TNFAIP3 Phe127Cys (rs2230926) is closely associated with the development of autoimmune diseases, including rheumatoid arthritis SLE, DM-1 type, aorto-arteritis [110]. Mutation in exon 3 (rs2230926 380T>G; F127C), leads to the loss of the ability of A20 to inhibit signaling of inflammatory cascades on the example of thyroid lymphoma [9] and rheumatoid arthritis, polymorphism in the TNFAIP3 gene is associated with Takayasu arteritis, while the signaling pathways STAT4, BANK1, BLK, and TNFSF4 remain intact [16]. Gaballah H A revealed a strong relationship between SNP rs2230926, rs5029939, rs5029937, and rs3757173 in the TNFAIP3 gene with clinical manifestations of SLE, and meta-analysis showed that rs5029939 and rs3757173 polymorphisms are associated with SLE in Asians, whereas rs2230926 and rs5029937 - only in Europeans [17]. Moreover, TNFAIP3 rs2230926, TNIP1 rs10036748 and BLK rs13277113 are associated with severe rheumatoid arthritis with high levels of SSR (citrulline peptide) and rheumatoid factor [18]. In the Lee MH review devoted to the mechanisms of autoimmunity and the significance of negative TNF-alpha regulators and caspase inhibitors, it was shown that A20 C103A cells - i.e. cells with TNFAIP3 rs2230926 gene polymorphism demonstrate an increase in the release of factors that attract neutrophils, as well as promoting the production of autoantibodies to citruline epitopes [19]. Adrianto I found that there is an independent genetic association of SLE and TNFAIP3 gene polymorphism in the European population - a mutation in the coding region of exon 3 (rs2230926 T>G; F127C, RefSeq: NP_006281) reduces the ability of A20 to block the NF-kappaB signaling pathway, which is accompanied by the implementation of inflammatory cascades [20].

Table 3. Distribution of candidate gene alleles in vitiligo patients

Alleles/genotypes	Main group		Control group		RR	Allele frequency by databases
	N=20	%	N=40	%		
rs2230926 F127C of gene TNFAIP3						
TT	17	85	39	97,5	0,14	
GG	0	0	0	0	0	
TG	3	15	1	2,5	6,9	
T	37	92,5	79	98,75	0,15	0,96-0,86
G	3	7,5*	1	1,25	6,4	0,04-0,14
rs753955178 P461P of gene TNFAIP3						
AA	18	90	39	97,5	0,23	
GG	0	0	0	0	0	
AG	2	10	1	2,5	4,3	
A	38	95	79	98,75	0,24	0,9971-0,9871
G	2	5*	1	1,25	4,15	0,0029- 0,0129
rs146534657 N102S of gene TNFAIP3						
AA	18	90	39	97,5	0,23	
GG	0	0	0	0	0	
AG	2	10	1	2,5	4,3	
A	38	95	79	98,75	0,24	0,998-0,988
G	2	5*	1	1,25	4,15	0,002- 0,012
rs142878172 IVS10-7C>A of gene REL						
CC	18	90	39	97,5	0,23	
AA	0	0	0	0	0	
CA	2	10	1	2,5	4,3	
C	38	95	79	98,75	0,24	0,9964-0,9924
A	2	5*	1	1,25	4,15	0,0036-0,00758
rs140572082 T433A of gene REL						
AA	18	90	40	100	0	
GG	0	0	0	0	0	
AG	2	10	0	0	0	
A	38	95	80	100	0	0,99946-0,9971
G	2	5	0	0	0	0,00054-0,0029

*-statistically significant relative to the control group at $p<0.05$.

Interesting results were obtained by Tejasvi T when studying the polymorphism of the TNFAIP3 gene (rs2230926 in exon 3 and rs610604 in intron 6) in psoriasis: these authors revealed that the response to TNF blockers (etanercept) depends on SNP, because the G-allele for SNP rs610604 of the TNFAIP3 gene and its haplotype with wild protective T-The rs2230926 allele are markers of a good response to treatment with TNF inhibitors in psoriasis, and a mutation in rs2230926 and the presence of an unfavorable G allele are markers of poor responsiveness to targeted therapy of psoriasis with TNF inhibitor Etanercep [21].

Our observations showed that all 3 carriers of the unfavorable mutant allele G rs2230926 of the TNFAIP3 gene had autoimmune thyroiditis with a high level of anti-TPO, while hypofunction of the thyroid gland was in 2 patients, and an isolated increase in TSH and anti-TPO at normal levels of T3 and T4 (euthyroid state) was in one patient. Before contacting us, none of the patients were examined for thyroid status. They had vitiligo in a generalized form, for a long time - more than 2 years, it was resistant to treatment.

There are significantly fewer indications about the polymorphism rs753955178 of the TNFAIP3 gene in the literature, there is no data on its connection with diseases, however, it has been shown that the reference allele is A, the variant allele is G. Polymorphism rs146534657 of the TNFAIP3 gene (TNFAIP3):c.305A>G (p.Asn102Ser) characterizes a missense mutation that occurs with a frequency of less than 1.3% (Global minor allele frequency (GMAF)=0.01298 (G)). There is no data in the literature on the connection of this polymorphism with vitiligo, but there is evidence of the connection of this polymorphism with autoimmune diseases - familial autoimmune syndrome, Behcet's disease (according to ARUP Laboratories, Molecular Genetics and Genomics, 2021) [15].

In our observations, the mutant unfavorable allele G SNP rs753955178 of the TNFAIP3 gene was found in the main group with a frequency of 5% versus 1.25% in the control group (χ2= 1.34; p>0.05), which is not statistically significant; the total population frequency of this allele is 0.29% (0.29-1.3%), i.e. the data obtained by us are consistent with the literature and indicate that the distribution of the mutant allele in vitiligo and in the population is not statistically significantly different. The relative risk of developing vitiligo in this case of carrying an unfavorable G allele for rs753955178 was 4.15, and requires more observations to establish the significance of this mutation for the development of vitiligo.

The results of the study of the single nucleotide polymorphism rs146534657 N102S of the TNFAIP3 gene were similar, RR was 4.15, and the frequency of the mutant allele was 5% versus 1.25 in the control (χ2= 1.34; p>0.05).

In 4 patients carrying mutant G alleles, according to TNFAIP3 gene polymorphisms (rs753955178 and rs146534657), the course of vitiligo was generalized, a distinctive feature was that in all these patients the disease was long-lasting - more than 5 years, relapsed and was poorly treatable. Thyroid status in 2 patients was euthyroid without increasing anti-TPO, and 2 patients had autoimmune thyroiditis with hypothyroidism.

Thus, as the results of the study of the TNF-alpha TNFAIP3 negative regulator gene have shown, single-nucleotide polymorphism rs2230926 with the carrier of an unfavorable G allele has a significant risk of developing vitiligo at RR=6.4 and in 100% of cases is combined with autoimmune thyroiditis, the carrier of unfavorable G alleles of two other TNFAIP3 gene polymorphisms (rs753955178 and rs146534657) is combined with autoimmune thyroiditis in 50% of observations. In general, carriers of unfavorable alleles of the three TNFAIP3 gene SNP studied have autoimmune thyroid pathology in 75% of cases, in 100% the disease is difficult to treat, lasts more than 2 years. This indicates that with a mutation in the gene of the negative regulator TNF-alpha, vitiligo has resistance to treatment, a tendency to progression, and proceeds against the background of an autoimmune process.

Our results of studying the polymorphism of proto-oncogenes from the REL gene family showed that the mutant allele A SNP rs142878172 IVS10-7C>A of the REL gene occurred with a frequency of 5% in the experimental group versus 1.25% in the control, and the population frequency of this allele is 0.36% -0.76%. Statistical data processing showed that the differences between the experimental and control groups in our studies were not significant (p>0.05), however, a significant difference was revealed between the frequency of the unfavorable allele A rs142878172 in the experimental group and the population frequency - 5% vs. 0.36% (χ2= 4,127328831, p<0.05), which indicates some significance of this SNP in the development of vitiligo, since the RR was 4.15. The course of vitiligo in 2 patients with this polymorphism was acrofacial in 1 case and generalized - in the second case, the duration of the disease was 5.5 years and 3.2 years, respectively, in a patient with generalized vitiligo there were signs of disease progression. The status of the thyroid gland was without features, 1 patient was diagnosed with IgG H.pillory and chronic cholecystitis, overweight.

When studying the rs140572082 T433A polymorphism of the REL gene, we identified a mutant G allele in 2 patients of the experimental group (5%), and in no case in the control, whereas the population frequency of this allele according to literature data is 0.054%-0.2%. Comparing our data with the general population, we note that the occurrence of the mutant allele was in 100 times higher, and the lack of detection of this allele in the control group indicates a small number of observations.

In two patients with the presence of the mutant allele G SNP rs140572082, vitiligo was generalized, one of the patients had IgG candida, and a secondary immunodeficiency was detected in the immunogram. I drew attention to the fact that mutant alleles were detected in this patient for both studied polymorphisms of the REL gene.

The information we have obtained regarding the significance of the REL SNP gene needs additional research, which is likely to confirm the significance of mutations in the REL gene for the development of vitiligo, given the universality of the NFkB signaling pathway regulated by this gene for the realization of apoptosis, oxidative stress and the synthesis of pro-inflammatory cytokines. Note that there is a lot of data in the literature on the importance of these proto-oncogenes for the regulation of proinflammatory signaling pathways.

This proto-oncogene is localized in chr2:60921756 (GRCh38.p13), is a regulator of the signaling pathway of transcription factor NFkB, an inhibitor of apoptosis [15]. For SNP REL rs142878172, the reference allele is C, variant alleles are A, G, T (C>A/C>G/C>T). The frequency of occurrence of alleles is less than 0.3% (A=0.003684 (975/264690, TOPMED; A=0.003805 (533/140090, GnomAD); A=0.00758 (376/49590,

ALFA), which indicates the goodness of this allele, since it has taken root in the population [15]. For SNP REL rs140572082, the reference allele is A>G, while the frequency of the mutant G allele is very low, amounting to less than 0.05% - MAF:G=0.00054/110 (ALFA); G=0.000214/30 (GnomAD); G=0.000223/1 (Estonian); G=0.000305/24 (PAGE_STUDY); G=0.000325/86 (TOPMED); G=0.002912/732 (GnomAD_exomes); G=0.003296/400 (ExAC); G=0.004593/23 (1000Genomes); G=0.00463/1 (Qatari); G=0.00463/1 (Vietnamese); A=0.5/5 (SGDPPRJ), which indicates its probable poor quality. REL genes are expressed in all tissues, because they regulate (suppress) apoptosis, modulate lymphopoiesis [181]. Their expression is most pronounced in the intestine (4.6), spleen (3.6); blood (3.5), lungs (3.4), nervous system (3.3), heart (3.2), lymph nodes (3.1), muscles (3.1), liver (3.1), kidneys (3.1), bone marrow (3.0), skin (2,9), pancreas (2,7), bones (2,3), stomach (2,3), eyes (2,3), thyroid gland (2,1) (ProteomicsDB, MaxQB). Diseases such as immunodeficiency and B-cell lymphoma are associated with REL genes.

The presence of drugs that modulate REL genes is attractive. It has been shown that triptolide is an inhibitor of IL-2/MMP-3, MMP-7 and MPP-19; the drug JSH-23 is an inhibitor of NFkB, also inhibitors of REL genes are chloramphenicol, cyclosporine, dexamethasone, diethylstilbestrol, nitric oxide [22]. With the help of these means, it is possible to prevent excessive apoptosis caused by mutation of REL genes and violations of NFkB signaling. TNF-a inhibitors, drugs that block the biological activity of TNF-alpha in circulation and at the cellular level, such as chimeric (infliximab) and human (adalimumab) monoclonal antibodies to TNF-a and etanercept, can be potentially effective with TNF-3 gene mutations [4].

Our results complement the understanding of the importance of pro-inflammatory genes in the pathogenesis of vitiligo in the Uzbek population: for example, Yakubova A. showed that SNP G-197A of the IL-17 gene is an independent marker and is associated with a higher level of IL-17 in vitiligo, but the significance of this SNP in vitiligo requires further research. We also identified a high level of pro-inflammatory and pro-apoptotic cytokines in patients with vitiligo and analyzed these indicators depending on the genetic polymorphism of the REL and TNFAIP3 genes.

Our results showed that the average TNF-alpha level was higher than the reference interval and the control group data, which proves the contribution of both inflammation and apoptosis to the implementation of depigmentation in vitiligo.

We focused our attention on the level of TNF-alpha and IL-21 precisely in those patients who had an unfavorable allele for the SNP genes REL and TNFAIP3 studied by us. The result showed that their concentrations of these cytokines were significantly higher than among the other patients of the main group (Table 4).

Table 4. Cytokine indicators in patients with vitiligo

Indicator	Reference interval	Control group (n=22)	Main group (n=37)	Patients-carriers of mutant alleles (n=10)
TNF-alpha, pg/ml	0-5,9	0,99± 0,1	8,63± 1,03*	12,1± 0,3*
IL-21, pg/ml	0,7 ±0,1	0,33± 0,01	1,82± 0,91	1,62± 0,02

Note that, *-statistically significant relative to the control group at $p<0.05$.

4. Conclusion

1. Among the concomitant diseases in patients with vitiligo, the following have a higher frequency and significantly increase the risk of developing vitiligo: hypo /hyperthyroidism (RR=17.3), autoimmune thyroiditis (RR=10.5), anemia (RR=6.5), liver and biliary tract diseases (RR=2.4), the presence of helminthic invasion (RR=6.7) and allergies (RR=2.6), and the presence of obesity and overweight can be considered a general unfavorable background for both the development of vitiligo and for the control group as a whole.
2. The presence of the mutant allele G rs2230926 F127C of the TNFAIP3 gene is statistically significant in the development of vitiligo at RR=6.4, associated with autoimmune thyroiditis, and unfavorable alleles SNP rs753955178 P461P and rs146534657 N102S of the TNFAIP3 gene occur with the same frequency both in vitiligo (5%) and in the general population (0.2-1.2%), $p>0.05$, but their carriage is combined with autoimmune thyroiditis in 50% of cases, and in general, carriers of unfavorable alleles of the three

TNFAIP3 gene SNPs studied have autoimmune thyroid pathology in 75% of cases, in 100%, the disease is difficult to treat, lasts for more than 2 years.

3. The presence of mutant unfavorable alleles of polymorphism of the REL gene occurred in patients with vitiligo in the form of a heterozygous genotype, the frequency of their occurrence did not significantly differ from that in the control group (5% vs. 1.25%), but was 100 times higher than the general population (5% vs. 0.36%) for rs142878172 and 5% vs. 0.054% for rs140572082 accordingly, it was associated with a poor result of vitiligo treatment.
4. The level of TNF-alpha and IL-21 in carriers of unfavorable alleles of the REL and TNFAIP3 genes is significantly higher than among other patients of the main group with vitiligo.

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